IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Patent Application of:
David F. Muir

Application No.: 10/812,776 — Confirmation No.: 4996

Filed: March 29, 2004 — Art Unit: 1657

For: Materials and methods for nerve grafting, selection of nerve grafts, and in vitro nerve tissue culture — Examiner: V. Afremova

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APPEAL BRIEF

This Appeal Brief is submitted in accordance with 37 C.F.R. § 41.37 in furtherance of the Notice of Appeal filed March 21, 2008, in support of the appeal from rejection of the pending claims in the above-identified application.

Appellant authorizes the Commissioner to charge $ 255.00 to cover the fee for filing this Appeal Brief as specified in 37 C.F.R. §41.20(b)(2). Appellant believes that no other fees are due. However, please consider this a conditional petition for the proper extension, if one is required, and authorization to charge any additional fees that may be due for further extensions of time or any other purpose associated with this submission, or credit any overpayment, to Appellant's undersigned counsel's deposit account number 07-1700 with reference to docket number AXO-003C1.

REAL PARTY IN INTEREST

The real parties in interest are the owner of the present application, the University of Florida Research Foundation, Inc., and the exclusive licensee of this application, AxoGen, Inc.

RELATED APPEALS AND INTERFERENCES

Appellant wishes to bring to the Board's attention a co-pending appeal in U.S.S.N. 10/218,315, which has been assigned Appeal No. 2008-3327. In the '3327 appeal, the Examiner is the same Examiner in the present appeal. The '315 patent application in the '3327 appeal and the patent application in the present appeal are commonly owned, have common subject matter, and claim priority to a common provisional patent application, U.S.S.N. 60/311,870.

Except for the '3327 appeal, Appellant is not aware of any other appeals, interferences, or judicial proceedings that are related to, directly affect, or will be directly affected by or have a bearing on the Board's decision in the present appeal.

STATUS OF CLAIMS

The application as filed contained 115 claims. New claims 117-123 were introduced during prosecution and claims 2-5, 24-29, 41, and 57-116 were canceled during prosecution. Claims 1, 6-23, 30-40, 42-56, and 117-123 remain pending, have been rejected, and are the subject of this appeal.

STATUS OF AMENDMENTS

No amendments have been filed subsequent to the Office Action mailed on January 30, 2008 (the "1/30/08 Office Action").

SUMMARY OF CLAIMED SUBJECT MATTER

The art has recognized that nerves can be predegenerated *in vivo* to support nerve regeneration.[1] As mentioned in the specification, it is impractical to create predegenerated nerves in humans because it would involve nerve injury followed by a period of survival *in vivo* to allow tissue degeneration.[2] In degeneration, cellular and molecular mechanisms act to enhance the growth-promoting properties of the basal lamina, which then retains the ability to stimulate nerve regeneration after cellular elements have been killed.[3] *In vitro* predegeneration, however, is practical and results in a substantial increase in the growth-promoting ability of acellular nerve grafts.[4] An example of such *in vitro* predegeneration involves methods that include predegenerating the nerve tissue by *in vitro* culturing which, following engraftment, improves the ability of regenerating axons to traverse the interface between the graft and host nerve tissue.[5]

[1] Page 6, lines 11-13 of the specification as filed.
[2] Page 6, lines 13-15 of the specification as filed.
[3] Page 7, lines 21-24 of the specification as filed.
[4] Page 7, lines 24-26 of the specification as filed.
[5] Page 27, lines 16-20 of the specification as filed.

In one aspect, the present invention as recited in independent claim 1 relates to a method for preparing a nerve graft suitable for implantation.[6] The chondroitin sulfate proteoglycan (hereinafter "CSPG") of a nerve graft that includes a nerve tissue segment is degraded by *in vitro* culturing, while maintaining an intact basal lamina tube structure of the nerve graft.[7] The CSPG degradation enhances post-implantation axonal traversal of an interface between the nerve graft and host nerve tissue.[8] In addition, the nerve graft is rendered acellular by killing cells in the nerve graft.[9]

In another aspect of the invention as recited in independent claim 38, the invention relates to a method for preparing a nerve graft suitable for implantation.[10] The CSPG of a nerve graft that includes a nerve tissue segment is degraded by *in vitro* culturing, while maintaining an intact basal lamina tube structure of the nerve graft.[11] The CSPG degradation enhances post-implantation axonal traversal of an interface between the nerve graft and host nerve tissue.[12] The culturing conditions can include a temperature within the range of about 10° C to about 37° C for a period of time within the range of about 24 hours to about 96 hours.[13] In addition, the nerve graft is rendered acellular by killing cells in the nerve graft.[14]

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[6] Page 9, lines 14-16 of the specification as filed.
[7] Page 8, line 16 to page 9, line 2 and page 27, lines 16-23 of the specification as filed.
[8] Page 27, lines 17-20 of the specification as filed.
[9] Page 28, lines 15-18 of the specification as filed.
[10] Page 9, lines 14-16 of the specification as filed.
[11] Page 8, line 16 to page 9, line 2 and page 27, lines 16-23 of the specification as filed.
[12] Page 27, lines 17-20 of the specification as filed.
[13] Page 28, lines 3-5 and 9-10 of the specification as filed.
[14] Page 28, lines 15-18 of the specification as filed.

GROUNDS FOR REJECTION TO BE REVIEWED ON APPEAL

The issues on appeal are as follows.

1. Whether claims 1, 6-23, 30-40, 42-56, and 117-123 are definite under 35 U.S.C. § 112, second paragraph.

2. Whether claims 1, 6-15, 17-21, 30-40, 42-51, 53-56, 117-120, and 122-123 are patentable under 35 U.S.C. § 102(b) over La Fleur *et al.* (1996), J. Exp. Med. 184: 2311-26 (hereinafter "La Fleur").

3. Whether claims 1, 6-15, 17-23, 30-40, 42-56, and 117-123 are patentable under 35 U.S.C. § 102(b) over Lassner *et al.* (1995), J. Reconstruct. Microsurg. 11(6): 447-453 (hereinafter "Lassner").

4. Whether claims 1, 6-15, 17-21, 30-32, 34-40, 42-45, 47-51, 53-56, 119, and 122-123 are patentable under 35 U.S.C. § 102(e) over U.S. Patent No. 6,448,076 by Dennis *et al.* (hereinafter "Dennis").

5. Whether claims 1, 6-23, 30-40, 42-56, and 117-123 are patentable under 35 U.S.C. § 103 over Dennis, LaFleur, Ide *et al.* (1983), Brain Res. 288: 61-75 (hereinafter "Ide"), and Evans *et al.* (1994), Progress Neurobiol. 43: 187-233 (hereinafter "Evans").

ARGUMENT

For the reasons that follow, Appellant respectfully submits that the pending claims are patentable. Appellant's claims are directed to a method for preparing a

nerve graft. The active steps in the method include degrading CSPG, which is achieved by *in vitro* culturing of a nerve graft comprising a nerve tissue segment. This active step results in enhanced post-implantation axonal traversal of an interface between the nerve graft and host nerve tissue relative to a nerve graft in which chondroitin sulfate proteoglycan was not degraded.

The Examiner misses the fact that Appellant's claims are directed to a method for preparing a nerve graft with an active step of degrading CSPG. Instead, she focuses solely on 'culturing.' In fact, it appears as if the Examiner ignores all other elements in Appellant's claims. As explained below, however, Appellant does not claim to have invented culturing; rather, the claims recite a method in which culturing is a necessary but by no means sufficient step toward preparing nerve grafts capable of post-implantation growth and function.

I. Rejection of claims 1, 6-23, 30-40, 42-56, and 117-123 under 35 U.S.C. § 112, second paragraph.

Claims 1, 6-23, 30-40, 42-56, and 117-123 stand rejected under 35 U.S.C. § 112, second paragraph. In the most recent Office Action, the Examiner alleges that "[t]he limitations [in independent claims 1 and 38] such as 'degrading CSPG' and 'enhancing post-implantation' are the intended effects of '*in vitro* culturing' as claimed. The culturing step is generic as claimed."[15] This is incorrect and goes to the heart of the Examiner's fundamental misunderstanding of Appellant's claims.

[15] 1/30/08 Office Action, page 2.

Independent claims 1 and 38 specifically recite, in pertinent part, "degrading, by *in vitro* culturing, chondroitin sulfate proteoglycan of a nerve graft. . . , thereby enhancing post-implantation axonal traversal of an interface between the nerve graft and host nerve tissue[.]"[16] Clearly, the active step recited in the claims is degrading CSPG. The step is not *in vitro* culturing, as alleged by the Examiner. The active degrading step recited in the claims is achieved by *in vitro* culturing-specific CSPG degradation, and not by any culturing procedure generally, as the Examiner mistakenly alleges.

The Examiner further alleges that "[n]o treatment agents and/or conditions are recited in the claims. Thus, it is uncertain what 'degrading' and/or 'enhancing' treatments are encompassed in the method for preparing a nerve graft."[17] With this rejection, the Examiner reveals her misreading of the claims as requiring separate steps of degrading, culturing, and enhancing. But the claims say no such thing. Rather, the claims recite degradation of CSPG by means of culturing, which enhances post-implantation axonal traversal. And the specification provides ample teaching regarding the culturing conditions required to degrade CSPG:

> The present invention also concerns methods of culturing nerve tissue for implantation into a human or animal. The culture methods of the subject invention involve "predegenerating" the nerve tissue *in vitro*, which, following engraftment, improves the ability of regenerating axons to traverse the interface between the graft and host nerve tissue. Without being bound by theory, the culturing methods of the subject invention allow the living nerve cells to express CSPG-degrading enzymes and

[16] Appellant's claims 1 and 38.
[17] 1/30/08 Office Action, page 2.

promote Schwann cell proliferation, as would occur naturally *in vivo* during the remodeling process of nerve degeneration.[18]

Accordingly, it is the Examiner's misguided disaggregation of the claims, rather than the claims themselves, that is improper.

II. Rejection of claims 1, 6-15, 17-21, 30-40, 42-51, 53-56, 117-120, and 122-123 under 35 U.S.C. § 102(b) over La Fleur.

A. La Fleur Neither Discloses Nor Suggests the Claimed Method

Claims 1, 6-15, 17-21, 30-40, 42-51, 53-56, 117-120, and 122-123 stand rejected under 35 U.S.C. §102(b) over La Fleur. But La Fleur fails to teach or suggest *a method for preparing a nerve graft suitable for subsequent implantation*, which is the subject of Appellant's invention and is required by Appellant's claims. Instead, La Fleur's objectives include identifying endogenous matrix metalloproteinases (MMPs) and the tissue inhibitors of MMPs (TIMPs) involved in repair after peripheral nerve injury. La Fleur's objectives also include determining the possibility that protection of basement membrane ***from*** proteolytic degradation is a relevant mechanism during repair of injury to nerve.[19]

In particular, La Fleur observes that, in response to crush injury, TIMP-1 is induced.[20] La Fleur also observes that TIMP-1 protects basement membrane type IV collagen from degradation by exogenous MMP-9 in cryostat sections of nerve *in*

[18] Appellant's specification, page 27, lines 16-23.
[19] La Fleur, page 2312, left column.
[20] La Fleur, abstract.

vitro.[21] La Fleur concludes with a proposal that in the proteolytic environment of injured nerve, TIMP-1 helps to preserve Schwann cell basement membrane during Wallerian degeneration, thus promoting axonal regrowth *in vivo*.[22]

Accordingly, La Fleur fails to teach or suggest a *method for preparing a nerve graft suitable for subsequent implantation*, because La Fleur simply is not concerned with and does not address this problem. La Fleur fails to teach or suggest a method of *degrading, by in vitro culturing, CSPG of a nerve graft* as required by Appellant's claims, instead proposing that protection of the basement membrane from degradation promotes axonal regrowth in injured nerves, *in vivo*. And indeed, in proposing preservation of the entire basement membrane and retention of CSPG, La Fleur is *antithetical* to Appellant's claims twice over: the claims require selective degradation of CSPG while maintaining the basal lamina tube structure.

B. La Fleur Does Not Utilize the Claimed Method Inherently

In the rejections, the Examiner references two analytical protocols in La Fleur[23] in which segments of nerves are removed from animals and cultured.[24] The first protocol includes removing crushed or control nerve segments from mice and culturing them for 24 hours. The second protocol includes removing control nerve segments and culturing them with macrophage- or growth factor-conditioned media to test the effect on TIMP-1 expression from the control nerve segments. Both protocols appear to

[21] Id.
[22] La Fleur, page 2323, right column.
[23] La Fleur, page 2312, right column, paragraphs 1-2.
[24] *See, e.g.*, 1/30/08 Office Action, page 3, third paragraph.

include a final step of removing the nerve segments from the culture and placing them in TRIzol, which is a concentrated solution of phenol.[25]

Based on these two protocols, the Examiner concludes that La Fleur comprises "identical active steps of culturing and killing nerve tissues under conditions as presently claimed. Thus, the cited reference anticipates the claimed invention."[26]

MPEP §2112.01 states in pertinent part:

> Where the claimed and prior art products are identical or substantially identical in structure or composition, or are produced by identical or substantially identical processes, a *prima facie* case of either anticipation or obviousness has been established. *In re Best,* 562 F.2d 1252, 1255, 195 USPQ 430, 433 (CCPA 1977). "When the PTO shows a sound basis for believing that the products of the Appellant and the prior art are the same, the Appellant has the burden of showing that they are not." *In re Spada,* 911 F.2d 705, 709, 15 USPQ2d 1655, 1658 (Fed. Cir. 1990). Therefore, the *prima facie* case can be rebutted by evidence showing that the prior art products do not necessarily possess the characteristics of the claimed product. *In re Best,* 562 F.2d at 1255, 195 USPQ at 433.

First, and contrary to the Examiner's contention, La Fleur's protocols are not identical to Appellant's claimed method. For example, in one protocol La Fleur places four-day-old crushed or control nerve segments from mice in culture to generate a conditioned medium. In the second protocol, La Fleur adds conditioned medium or growth factors to nerve segments in culture medium to see if the nerve segments alter their gene expression. Accordingly, the steps are not identical to Appellant's claims.

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[25] TRIzol Material Safety Data Sheet, Invitrogen, Carlsbad, California, last revised 9/8/06; IDS reference C5 in prosecution history.
[26] 1/30/08 Office Action, page 3, last paragraph.

Second, there is no indication or evidence that either of La Fleur's protocols yields a nerve tissue sample with an *intact basal lamina tube structure*, as required by the present claims; certainly La Fleur's protocols do not necessarily yield such tissue as articulated in MPEP §2112.01. La Fleur also describes the isolation of proteins from a tissue sample through the homogenization of the nerve segment.[27] As homogenization involves crushing and rendering the sample into a fine powder or slurry, this process also is unsuitable for a method for *preparing a nerve graft suitable for subsequent implantation*, and fails to *maintain an intact basal lamina tube structure*, as required by the present claims.

Third, any nerve tissue subjected to La Fleur's protocols could be harmful if implanted as a nerve graft. For example, these methods include, as a final step, submersion of the nerve tissue in TRIzol to extract nucleic acids from tissue samples.[28] As noted above, TRIzol is a solution of concentrated phenol. Phenol is a toxic and corrosive material.[29] Exposure to phenol is known to cause deep necrosis, cardiac dysrhythmias, metabolic acidosis, hyperventilation, respiratory distress, acute renal failure, renal damage, dark urine, methaemoglobinaemia, neurological effects (including convulsions), cardiovascular shock, coma and death.[30] Accordingly, the use

[27] La Fleur, page 2312, column 2, last paragraph.
[28] La Fleur, page 2312, column 2, second paragraph.
[29] *See, e.g. IPCS Environmental Health Criteria for Phenol (161)*, World Health Organization (publ. 1994, available at the web site, www.inchem.org/documents/ehc/ehc/ehc161.htm, last visited November 12, 2007, first draft by Montizan GK, printed in Finland, IDS reference C6 in prosecution history; Brooks and Riviere (1996) "Quantitative Percutaneous Absorption and Cutaneous Distribution of Binary Mixtures of Phenol and para-Nitrophenol in isolated Perfused Porcine Skin. Fundamental and Applied Toxicology" 32: 233-243, IDS reference C7 in the prosecution history.
[30] *See* IDS reference C6, particularly Section 1.7, in prosecution history.

of concentrated phenol is inconsistent with *preparing a nerve graft suitable for subsequent implantation.*

When faced with the argument that La Fleur's method of treating a nerve segment with TRIzol could be harmful if implanted as a nerve graft, the Examiner responded that "the final step of [Appellant's] claimed method involves 'killing' cells (claims 1 and 38) including 'killing' by generic 'chemical treatment' (claims 21 and 49, for example)."[31] But this is irrelevant, because whether or not the graft is rendered acellular with a chemical treatment, the claimed methods require that the nerve graft be suitable for subsequent implantation. This is not possible for nerve segments (such as La Fleur's) that have been soaked in a substance that is toxic if applied to the body.

Therefore, Appellant respectfully submits that La Fleur fails to teach or suggest each and every element of claims 1, 6-15, 17-21, 30-40, 42-51, 53-56, 117-120, and 122-123.

III. Rejection of claims 1, 6-15, 17-23, 30-40, 42-56, and 117-123 under 35 U.S.C. § 102(b) over Lassner.

Claims 1, 6-15, 17-23, 30-40, 42-56 and 117-123 stand rejected under 35 U.S.C. §102(b) over Lassner, which describes methods for preserving cellular viability in peripheral nerve grafts. The paragraph bridging columns 1 and 2 of page 448 of Lassner describes three experimental groups: nerve segments placed in cold storage at 4°C under ischemic conditions for periods of 14 hours, 32 hours, 72 hours, or 120 hours

[31] 1/30/08 Office Action, page 9, first paragraph.

(Groups A-H); normal animal controls having the nerve dissected, left in the animal, and subsequently sutured in the absence of extracorporeal pretreatment (Group K), and negative controls where nerves were subjected to repeated freezing and thawing to evacuate all viable cells (Group I). Therefore, the nerve sections were (1) removed from the animal and stored in cold, ischemic conditions prior to implantation, (2) left in the animal after nerve dissection and subsequently sutured, or (3) removed from the animal and simply rendered acellular prior to implantation.

These activities do not represent *in vitro* culturing, as required by Appellant's claims. The cold, ischemic conditions are stasis conditions and do not promote physiological activity. In Appellant's method, if there is no physiological activity, there is no *degradation of CSPG* and, thus, no *enhancement of post-implantation axonal traversal of an interface between the nerve graft and host nerve tissue.*[32] Thus, Lassner's stasis conditions are not culturing conditions as recited in Appellant's claims. Furthermore, leaving the severed nerve in the animal is not an *in vitro* treatment (it is an *in vivo* treatment) and, thus, cannot anticipate claim limitations that require culturing *in vitro.* In none of the three described dispositions of nerve segments does Lassner disclose or even suggest degrading CSPGs, axons or myelin by culturing a nerve graft *in vitro.*

The Examiner also alleges that a second series of experiments in Lassner may be relevant to Appellant's claims.[33] In Lassner's second series of experiments, nerve

[32] Appellant's specification, page 56, lines 17-19.
[33] Lassner, page 448, column 2, last paragraph; 1/30/08 Office Action, page 4, last paragraph; paragraph bridging pages 9-10.

grafts were prepared, dissected into small segments, placed in a culture dish containing Dulbecco's Modified Eagle Medium, and maintained at 5% CO_2/95% air for two days. The tissue segments were then evaluated morphologically, fixed with methanol at -18°C, and immunohistologically stained without any reimplantation. Thus, these experiments involve preparation of histological samples and do not describe a *method for preparing a nerve graft suitable for subsequent implantation* or a method for *enhancing the regenerative potential of a nerve graft*, as required by Appellant's claims, nor are such samples inherently suitable for reimplantation. Indeed, methanol is a toxic substance which, according its Material Safety Data Sheet, "cannot be made non-poisonous."[34] Accordingly, the use of methanol is not compatible with preparing a nerve graft for subsequent implantation.

Additionally, the described conditions do not result in a graft that is structurally the same as the graft prepared from Appellant's claimed method. Specifically, there is no indication that Lassner's samples comprise degraded CSPG or have an intact basal lamina tube structure. Nor does Lassner's histological preparation method result in the enhancement of *post-implantation axonal traversal of an interface between the nerve graft and host nerve tissue*. It simply cannot, because histological samples (i.e. thin transverse sections of the nerve for microscopic evaluation) do not function as nerve grafts.

Therefore, Appellant submits that Lassner fails to teach or suggest each and every element of claims 1, 6-15, 17-23, 30-40, 42-56 and 117-123.

[34] Methanol Material Safety Data Sheet, Fisher Scientific, last revised 6/29/07; IDS reference C8 in prosecution history.

IV. *Rejection of claims 1, 6-15, 17-21, 30-32, 34-40, 42-45, 47-51, 53-56, 119, and 122-123 are patentable under 35 U.S.C. § 102(e) over Dennis.*

Claims 1, 6-15, 17-21, 30-32, 34-40, 42-45, 47-51, 53-56, 119, and 122-123 stand rejected under 35 U.S.C. §102(e) over Dennis. Dennis describes a method of acellularization and does not include a *degradation step by in vitro culturing*, as required by Appellant's claims. Briefly, rat peripheral nerve segments are surgically removed, pinned at slack length within a culture dish, and immediately submersed in Dulbecco's Phosphate Buffered Saline (hereinafter "PBS"). Then, the acellularization method is carried out at room temperature.[35] These acellularized nerve grafts reportedly support axonal regeneration and allow end-organ reinnervation.[36] With regard to independent claims 1 and 38, Dennis does not describe a selective *degrading step by in vitro culturing*. In Dennis, the nerve is placed in PBS and then acellularization is carried out. There is no *in vitro culturing*.

Furthermore, Dennis fails to teach or suggest *degrading CSPG of the nerve graft while maintaining an intact basal lamina structure*. In fact, Dennis teaches just the opposite. Dennis states that "the acellularization method of the present invention ... preserves the basal lamina in order to maintain the appropriate molecular signals and adhesion molecules to enhance axonal regeneration."[37] Thus, while Dennis may describe maintenance of the basal lamina, Dennis fails to teach degrading CSPG. And while Dennis does mention "a method of acellularization which removes the cellular

[35] Dennis, column 3, lines 34-50.
[36] Dennis, column 6, lines 21-24.
[37] Dennis, column 6, lines 7-12.

elements from peripheral nerve tissue while leaving the endoneurial architecture intact,"[38] the resulting graft is described as having the cells removed. It is not described as having *degraded CSPG* as required by Appellant's claims.

Still further, Dennis fails to teach or suggest *enhancing post-implantation axonal traversal of an interface between the nerve graft and host nerve tissue*. As explained in the present application, culturing conditions under certain circumstances activate CSPG-degrading enzymes and/or involve addition of CSPG-degrading enzymes.[39] These conditions enhance post-implantation axonal traversal of an interface between the nerve graft and host nerve tissue. Although Dennis may describe nerve grafts that support axonal regeneration, it does not follow that either degradation of CSPG or enhancement of post-implantation axonal traversal of an interface between the nerve graft and host nerve tissue occur. This is demonstrated, for example, in Appellant's Example 18 and Figure 20A, which indicate that axonal regeneration into acellular nerve grafts is enhanced by *in vitro* predegeneration, but that axonal growth occurred within the basal lamina tubes in both the predegenerated and control (i.e., acellular but not predegenerated) conditions. Thus, it simply cannot be inferred from Dennis' description of a graft supporting axonal regeneration that CSPG is degraded or that post-implantation axonal traversal of an interface between the nerve graft and host

[38] Dennis, column 2, line 22-24.
[39] *See, e.g.,* Appellant's specification, page 27, lines 20-23; page 28, lines 18-20; and Example 3.

nerve tissue is enhanced, and the Federal Circuit has repeatedly held that the relevance of a reference cannot be predicated on "mere conjecture."[40]

The chemicals used in the graft preparation method described in Dennis also damage the graft's *tubular structure* that is maintained in Appellant's claimed invention. Accordingly, Dennis' methods are not compatible with a method for *preparing a nerve graft suitable for subsequent implantation* as required by the Appellant's claims. For example, Dennis describes submersing tissue in a solution of glycerol, in a solution of sodium deoxycholate, and in a solution containing TRITON-X 100.[41] Glycerol has been investigated for use in preserving testicular tissue as an option in fertility preservation for pre-pubertal boys who will lose spermatogenic cells as a result of chemotherapy.[42] In the study, when glycerol was used, the structure of the basal compartment of the tubules was severely damaged. The ultrastructure of the cryopreserved samples as revealed by high-resolution microscopy confirmed the findings. While glycerol is a widely used cryoprotectant for cryopreservation of cells, results indicate that it is not appropriate for preservation of tissues where structural maintenance is critical, such as with maintenance of basal lamina tube structure.

Previous researchers have studied the efficacy of using sodium deoxycholate and TRITON-X 100 in graft preparation. *See* Hudson *et al.* (2004) Tissue Engineering, 10 (9-10):1346-58, IDS reference C10 in prosecution history (hereinafter "Hudson"); Sondell *et al.* (1998) Brain Res. 795:44-54, IDS reference 11 in prosecution history,

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[40] *In re Robinson W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851, 105 S.Ct. 172 (1984).
[41] Dennis, columns 3-4 and claims 1 and 5, for example.
[42] Keros *et al.* (2005) Human Reproduction, 20(6): 1676-1687, IDS reference C9 in prosecution history.

(hereinafter "Sondell"). For example, Hudson, like Dennis, focused on the chemical acellularization of grafts but did not describe a degradation step via *in vitro* culturing. Briefly, Hudson sought to develop an optimized chemical acellularization method specifically for peripheral nerve tissue. Various detergents, concentrations and contact times were evaluated based on their ability to clear out cells and cellular debris while maintaining the critical extracellular matrix (or basal lamina) structure. The "optimized" protocol that was determined was then compared to existing acellularization methodologies, including Sondell's method, which, like Dennis' method, employs sodium deoxycholate and TRITON-X 100. Hudson found that the method employing sodium deoxycholate and TRITON-X 100 was effective at clearing out cells and debris but destroyed the basal lamina tube structure.[43]

In the most recent Office Action, the Examiner responds to the fact that Dennis's chemical acellularization treatments potentially compromise an *intact basal lamina structure* by stating that "the images presented by the reference by Sondell demonstrate preserved tubular structure or preserved basal lamina tubes of the nerve segments treated with Triton X-100, for example: see Fig. 1 and Fig. 5 in the reference by Sondell."[44] The Examiner cites no basis for this conclusion, nor could she. Hudson teaches destruction of the basal lamina tube structure using the method of Sondell. For example, Hudson reports, "The Sondell treatment appears to fragment the basal laminae."[45] Thus, Sondell simply does not stand for the cited proposition, and Dennis

[43] Hudson, Figure 5, page 1356 column 1, last sentence, and column 2, first paragraph.
[44] 1/30/08 Office Action, paragraph bridging pages 10-11.
[45] Hudson, page 1353, right column.

does not teach a method for preparing a nerve graft suitable for subsequent implantation.

Therefore, Appellant submits that Dennis fails to teach or suggest each and every element of claims 1, 6-15, 17-21, 30-32, 34-40, 42-45, 47-51, 53-56, 119, and 122-123.

V. *Rejection of claims 1, 6-23, 30-40, 42-56, and 117-123 under 35 U.S.C. § 103 over Dennis, La Fleur, Ide, and Evans.*

Claims 1, 6-23, 30-40, 42-56 and 117-123 stand rejected under 35 U.S.C. §103(a) over Dennis, La Fleur, Ide and Evans. As explained above, both Dennis and La Fleur fail to describe *degrading, by in vitro culturing, CSPG of a nerve graft*. In addition, the methods of both Dennis and La Fleur result in grafts that are structurally different from grafts produced in accordance with the present claims, and also involve chemicals that simply are not compatible with a *method of preparing a nerve graft suitable subsequent implantation*. Ide and Evans fail to cure the deficiencies of Dennis and La Fleur, alone or in combination.

Ide describes the influence of the basal lamina and living Schwann cells on nerve regeneration. The methods described in Ide focus solely on acellularization and do not involve selectively *degrading, by in vitro culturing, CSPG while maintaining an intact basal lamina tube structure*, as required by the present claims. In fact, Ide teaches away from the claimed invention by suggesting that there may be "specific substances

responsible for supporting the regenerating axons" within the inner surface of the basal lamina, thereby stressing the importance of its complete preservation.[46]

Evans is directed to *in vivo* predegeneration. *In vivo* predegeneration involves an experimental model used to investigate the mechanisms and potential benefits of Wallerian degeneration, a process that occurs naturally in the body prior to nerve regeneration. The Evans technique involves transecting the donor nerve and leaving the segment within the animal to "predegenerate" for a period of time prior to harvesting to allow Wallerian degeneration to take place with subsequent proliferation of Schwann cells and removal of myelin debris. The "predegenerated" grafts are then used for implantation in a different recipient animal; outcomes were compared with freshly recovered nerves used as a graft (i.e., nerves that had not been predegenerated *in vivo*).[47] Evans does not describe or suggest that a nerve graft can be predegenerated *in vitro*, that is, selectively degenerated (e.g., in a dish) while maintaining the basal lamina tube structure as required by Appellant's claims. In fact, Evans teaches away from Appellant's claimed method in reporting that predegeneration has no clinical relevance.[48]

As explained above, Dennis and La Fleur do not describe *in vitro culturing* as required by Appellant's claims. Neither Ide nor Evans, alone or in combination, cures at least this deficiency. Accordingly, there is no reason why one of skill in the art would modify the teachings of Dennis and/or La Fleur as the Examiner suggests; there

[46] Ide, abstract, page 71, column 1, last paragraph through column 2, first paragraph.
[47] Evans, page 209, column, 1 paragraphs 1-2.
[48] Evans, page 212.

is no reasonable expectation of success were such modification to be undertaken; and most importantly, Dennis, La Fleur, Ide and/or Evans fail to teach Appellant's claimed invention even when combined as the Examiner proposes. In fact, as discussed above, the references teach away from Appellant's claimed invention.

In view of the foregoing, Appellant respectfully requests that relief from the Examiner's misunderstanding.

VI. Conclusion

For all of the foregoing reasons, Appellant submits that the Examiner's rejections of the above-identified claims were erroneous, and reversal thereof is respectfully requested.

Respectfully submitted,

/Randall D. Morin/

Date: May 21, 2008

Randall D. Morin
Reg. No. 58,312
Goodwin | Procter LLP
Exchange Place
Boston, MA 02109
Tel. No. (617) 570-1657
Fax No. (617) 523-1231

CLAIMS APPENDIX

Listing of Claims:

1. A method for preparing a nerve graft suitable for subsequent implantation, the method comprising:

degrading, by *in vitro* culturing, chondroitin sulfate proteoglycan of a nerve graft comprising a nerve tissue segment while maintaining an intact basal lamina tube structure of the nerve graft, thereby enhancing post-implantation axonal traversal of an interface between the nerve graft and host nerve tissue relative to a nerve graft in which chondroitin sulfate proteoglycan was not degraded; and

rendering the nerve graft acellular by killing cells in the nerve graft.

2-5. (Cancelled)

6. The method according to claim 1, wherein said culturing of the nerve graft *in vitro* is for a period of time that achieves an increase in post-implantation axon ingress and extent of growth within the nerve graft relative to the nerve graft in which chondroitin sulfate proteoglycan was not degraded.

7. The method according to claim 1, wherein said culturing of the nerve graft *in vitro* is for a period of time within the range of about 24 hours to about 96 hours.

8. The method according to claim 1, wherein said culturing of the nerve graft *in vitro* is for a period of time within the range of about 24 hours to about 72 hours.

9. The method according to claim 1, wherein said culturing of the nerve graft *in vitro* is for a period of time of about 48 hours.

10. The method according to claim 1, wherein said culturing of the nerve graft *in vitro* is conducted at a temperature within the range of about 10° C to about 37° C.

11. The method according to claim 1, wherein said culturing of the nerve graft *in vitro* is conducted at a temperature within the range of about 30° C to about 37° C.

12. The method according to claim 1, wherein said culturing of the nerve graft *in vitro* is conducted at a temperature of about 37° C.

13. The method according to claim 1, wherein the nerve graft is an explant.

14. The method according to claim 1, wherein the nerve graft is mammalian tissue.

15. The method according to claim 1, wherein the nerve graft is mammalian tissue selected from the group consisting of human tissue, non-human primate tissue, porcine tissue, rodent tissue, and bovine tissue.

16. The method according to claim 1, wherein the nerve graft is human tissue.

17. The method according to claim 1, wherein the nerve graft is an autograft.

18. The method according to claim 1, wherein the nerve graft is an allograft.

19. The method according to claim 1, wherein the nerve graft is a xenograft.

20. The method according to claim 1, wherein rendering the nerve graft acellular by killing cells in the nerve graft occurs after culturing.

21. The method according to claim 1, wherein rendering the nerve graft acellular by killing cells in the nerve graft comprises a process selected from the group consisting of freeze-killing and chemical treatment.

22. The method according to claim 1, wherein said method further comprises freezing the nerve graft for storage.

23. The method according to claim 22, wherein said freezing is carried out after said culturing *in vitro*.

24-29. (Cancelled)

30. The method according to claim 1, wherein the nerve graft comprises peripheral nerve tissue.

31. The method according to claim 1, wherein said culturing comprises placing the nerve graft in contact with culture medium.

32. The method according to claim 31, wherein the culture medium comprises a defined medium.

33. The method according to claim 31, wherein the culture medium comprises a defined medium supplemented with serum.

34. The method according to claim 31, wherein the culture medium comprises undefined medium.

35. The method according to claim 31, wherein the culture medium comprises dulbecco's modified eagles' medium.

36. The method according to claim 1, wherein said method further comprises isolating the nerve graft from a mammal prior to said culturing of the nerve graft *in vitro*.

37. The method according to claim 1, wherein said method further comprises applying a tissue adhesive to the nerve graft .

38. A method for enhancing the regenerative potential of a nerve graft suitable for subsequent implantation, the method comprising:

degrading, by *in vitro* culturing, chondroitin sulfate proteoglycan of a nerve graft comprising a nerve tissue segment while maintaining an intact basal lamina tube structure of the nerve graft, thereby enhancing post-implantation axonal traversal of an interface between the nerve graft and host nerve tissue relative to an a nerve graft in which chondroitin sulfate proteoglycan was not degraded, wherein culturing conditions comprise a temperature within the range of about 10° C to about 37° C for a period of time within the range of about 24 hours to about 96 hours; and

rendering the nerve graft acellular by killing cells in the nerve graft .

39. The method according to claim 38, wherein said culturing of the nerve graft *in vitro* is for a period of time within the range of about 24 hours to about 72 hours.

40. The method according to claim 38, wherein said culturing of the nerve graft *in vitro* is for a period of time of about 48 hours.

41. (Cancelled)

42. The method according to claim 38, wherein said culturing of the nerve graft *in vitro* is conducted at a temperature within the range of about 30° C to about 37° C.

43. The method according to claim 38, wherein said culturing of the nerve graft *in vitro* is conducted at a temperature of about 37° C.

44. The method according to claim 38, wherein said culturing comprises placing the nerve graft in contact with culture medium.

45. The method according to claim 44, wherein the culture medium comprises defined medium.

46. The method according to claim 44, wherein the culture medium comprises defined medium supplemented with serum.

47. The method according to claim 44, wherein the culture medium comprises undefined medium.

48. The method according to claim 38, wherein rendering the nerve graft acellular by killing cells in the nerve graft occurs after culturing.

49. The method according to claim 38, wherein rendering the nerve graft acellular by killing cells in the nerve graft comprises a process selected from the group consisting of freeze-killing and chemical treatment.

50. The method according to claim 38, wherein the nerve graft is mammalian tissue.

51. The method according to claim 38, wherein the nerve graft is mammalian tissue selected from the group consisting of human tissue, non-human primate tissue, porcine tissue, rodent tissue, and bovine tissue.

52. The method according to claim 38, wherein the nerve graft is human tissue.

53. The method according to claim 38, wherein the nerve graft comprises peripheral nerve tissue.

54. The method according to claim 38, wherein the nerve graft is an autograft.

55. The method according to claim 38, wherein the nerve graft is an allograft.

56. The method according to claim 38, wherein the nerve graft is a xenograft.

57.-116. (Cancelled)

117. The method according to claim 1, wherein the nerve graft comprises central nervous system tissue.

118. The method according to claim 38, wherein the nerve graft comprises central nervous system tissue.

119. The method according to claim 38, wherein the nerve graft is an explant.

120. The method according to claim 38, wherein said method further comprises freezing the nerve graft for storage.

121. The method according to claim 120, wherein said freezing is carried out after said culturing *in vitro*.

122. The method according to claim 38, wherein said method further comprises isolating the nerve graft from a mammal prior to said culturing of the nerve graft *in vitro*.

123. The method according to claim 38, wherein said method further comprises applying a tissue adhesive to the nerve graft.

EVIDENCE APPENDIX

None.

RELATED PROCEEDINGS APPENDIX

None.